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EVALUATION OF ANTIEPILEPTIC ACTIVITY OF ETHANOL EXTRACT OF LEAVES OF GOSSYPIUM HERBACEUM IN MICE

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ABSTRACT

Aim: *The aim of the present study was to investigate antiepileptic activity of ethanol extract of Gossypium herbaceum (EEGH) in mice.* ***Method:*** *The antiepileptic activity of EEGH at 10, 30, and 100 mg/Kg, p.o. was evaluated by the convulsions induced in mice by maximum electroshock (MES), Pentylenetetrazole (PTZ) and Isoniazid (INH). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's t test.* ***Results and Discussion:*** *In MES and PTZ methods, EEGH (10, 30, and 100 mg/Kg) inhibited convulsions significantly potent than Diazepam and Phenobarbitone sodium (PS). In INH method, EEGH delayed the onset of convulsions less potent than Diazepam.* ***Conclusion:*** *In Present investigation, EEGH showed significant dose-dependent antiepileptic effect potent than Diazepam and PS.*

KEY WORDS

Epilepsy, Pentylenetetrazole, Isoniazid

INTRODUCTION

Epilepsy is a serious neurological disorder, which does not have any boundaries such as age, race, social class or nationality. The incidence of the disease in developing countries is higher than that in developed countries and is reported to be 57 per 1000.

Drug therapy of epilepsy with currently available anti-epileptic drugs (AED) is associated with dose-related side effects and chronic toxicity that involves virtually every organ system. It can be well imagined that all the above-mentioned problems with the current AED therapy of epilepsy are more prevalent in underdeveloped countries due to lack of facilities for proper diagnosis, treatment and monitoring serum levels of AED.

Different types of epileptic seizures have varied susceptibility to currently available AED and on the whole approximately two thirds of the patients with epilepsy can have remission of seizures[1]. There is a pressing need for further research in the field of pharmacotherapy of epilepsy to find drugs with lesser adverse effects. Search for anti-epileptic agents has made man turn to alternative sources i.e., exploitation of medicinal plants.

Leaves of *Gossypium herbaceum* were reported to possess antiepileptic activity[2]. Hence, the present study was aimed to evaluate antiepileptic activity of ethanol extract of *Gossypium herbaceum* (EEGH) in mice.

MATERIALS AND METHODS

Plant Material

Leaves of *Gossypium herbaceum* were collected from Nellutla, Andhra Pradesh, India. It was identified and authenticated by Prof. V. S. Raju, department of Botany, Kakatiya University. The plant specimen was deposited at Kakatiya University Herbarium (KUW), Warangal with voucher number 1865.

Preparation of the extract

The fresh leaves of *Gossypium herbaceum* were collected and washed under running tap water. They

were shade dried at room temperature. Then dried leaves were made in to coarse powder. The powder was passed through a 60 No mesh sieve. Then ethanol extract was prepared by cold maceration[3].

Qualitative analysis

The extract was subjected to phytochemical screening by using different qualitative tests[4].

Acute toxicity study

Acute toxicity study will be performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Cooperation and Development, as per 420 guidelines (OECD)[5].

Evaluation of Antiepileptic activity

Maximum electroshock (MES) in mice

Five groups of six male Swiss albino mice (25 – 30g) were used. The test was started one hour after oral treatment with the test compound (EEGH 10, 30, and 100 mg/Kg, p.o.) or the vehicle or the standard (Diazepam 3 mg/Kg, p.o.). An apparatus with corneal electrodes was used to deliver the stimuli. The intensity of the stimulus is dependent on the apparatus, eg: 30mA, 50Hz for 0.2 sec has been used. The onset and the duration of tonic himb extension was recorded and percentage of inhibition of seizures relative to controls was calculated[6].

Pentylenetetrazole (PTZ)-induced convulsions in mice

Control group received vehicle, test group received EEGH (10, 30, and 100 mg/Kg, p.o.) and standard group received Phenobarbitone sodium, (40 mg/Kg, i.p.). Convulsions were induced by administering PTZ (75 mg/Kg, i.p.), 1hr after EEGH and 15 min after PS and diazepam administration. The onset and the duration of convulsions were recorded, and percentage inhibition was calculated[7].

Isoniazid (INH)-induced convulsions in mice

Control group received vehicle, test group received EEEGH (10, 30 and 100 mg/Kg, p.o.) and standard group received Diazepam, (4 mg/Kg, i.p.). Convulsions were induced by administering INH (300 mg/Kg, s.c.), 1hr after drug administration. The onset time of convulsions was recorded[8].

STATISTICAL ANALYSIS

The data were analyzed by using one-way analysis of variance (ANOVA), followed by Dunnett's test. *p<0.05 was considered as significant.

RESULTS AND DISCUSSION

Percentage yield:

The ethanol extract of leaves of *Gossypium herbaceum* was prepared by following maceration. After preparing the extract, the percentage yield was found to be 31.65%.

Qualitative analysis

In preliminary phytochemical screening ethanol extract shows presence of steroids, alkaloids, flavonoids and tannins.

Acute toxicity study

Toxicity was found at 1000 mg/kg, p.o. and was found to be safe upto 600 mg/kg, p.o. So, 10, 30 and 100 mg/Kg were the doses selected for the study.

Evaluation of Antiepileptic activity

Maximum electroshock (MES) in mice

The onset time of THLE for control group mice was 1.35±0.04 sec. EEGH treated mice showed the onset time as 1.96±0.11, 2.66±0.05 and 4.62±0.05 sec (p<0.01) respectively at the doses of 10, 30 and 100 mg/kg, p.o. The standard group mice (diazepam 3 mg/kg, p.o.) showed 2.46±0.08 sec (p<0.01).

The duration of THLE for control group mice is 118.91±1.99 sec. Albino mice which received EEGH showed the duration of 44.28±0.91, 38.38±0.91 and 32.06±0.59 sec (p<0.01) respectively at the doses of 10, 30 and 100 mg/kg, p.o. The standard group mice (diazepam 3 mg/kg, p.o.) showed 49.37±0.74 sec (p<0.01).

The percentage inhibition achieved in animals which received EEGH were 62.76% (10 mg/kg), 67.72% (30 mg/kg) and 73.04% (100 mg/kg) (p<0.01) respectively when compared to control group animals (Table 1).

Table 1: Effect of EEGH on maximal electroshock-induced convulsions in mice

Group (n=6)=6)	Treatment	Onset of THLE (sec)	Duration of THLE (sec)	Percentage inhibition of convulsions
I	DMSO	1.35±0.04	118.91±1.99	-
II	EEGH (10 mg/kg)	1.96±0.11**	44.28±0.91**	62.76**
III	EEGH (30 mg/kg)	2.66±0.05**	38.38±0.91**	67.72**
IV	EEGH	4.62±0.05**	32.06±0.59**	73.04**

Group (n=6)=6)	Treatment	Onset of THLE (sec)	Duration of THLE (sec)	Percentage inhibition of convulsions
	(100 mg/kg)			
V	Diazepam (3 mg/kg)	2.46±0.08**	49.37±0.74**	58.48**

EEGH: Ethanol extract of leaves of *Gossypium herbaceum*; Values are mean ± SD (n = 6). Statistical significance was determined by ANOVA, followed by Dunnett's t test; **P < 0.01.

The time of onset of THLE for the control group animals was very less when compared to the extract and standard treated animals. Duration of THLE for the control group animals was greater when compared to the extract and standard treated animals. Albino mice pretreated with EEGH at the doses of 10, 30 and 100 mg/kg were provided significant protection from convulsions induced by electroshock method in a dose-dependent manner.

Pentylenetetrazole (PTZ) induced convulsions in mice

The onset time of convulsions for control group mice was 7.43±0.11 min. Albino mice pretreated with EEGH at the doses of 10, 30 and 100 mg/kg, p.o. exhibited the onset time as 15.55±0.05, 20.59±0.22 and 25.51±0.25 min (p<0.01) respectively. Mice which received Phenobarbitone sodium (40 mg/kg, i.p.) showed onset time of 4.51±0.09 min (p<0.01).

The duration of convulsions for control group mice was 18.57±0.40 min. Mice pretreated with EEGH at the doses of 10, 30 and 100 mg/kg, p.o. exhibited the duration as 10.05±0.31, 6.23±0.07 and 2.05±0.03 min (p<0.01) respectively. Mice belonging to standard group (Phenobarbitone sodium, 40 mg/kg, i.p.) showed 9.24±0.09 min (p<0.01).

The percentage inhibition achieved in animals which received EEGH were 45.87%, 66.48% and 88.96% (p<0.01) respectively at the doses of 10, 30 and 100 mg/kg when compared to control group animals (Table 2).

Table 2: Effect of EEGH on Pentylenetetrazole (PTZ)-induced convulsions in mice

Group (n=6)=6)	Treatment	Onset of convulsions (min)	Duration of convulsions (min)	Percentage inhibition of convulsions
I	DMSO	7.43±0.11	18.57±0.40	-
II	EEGH (10 mg/kg)	15.55±0.05**	10.05±0.31**	45.87**
III	EEGH (30 mg/kg)	20.59±0.22**	6.23±0.07**	66.48**
IV	EEGH (100 mg/kg)	25.51±0.25**	2.05±0.03**	88.96**
V	Phenobarbitone sodium (40 mg/kg, i.p.)	4.51±0.09**	9.24±0.09**	50.23**

EEGH: Ethanol extract of leaves of *Gossypium herbaceum*; Values are mean ± SD (n = 6). Statistical significance was determined by ANOVA, followed by Dunnett's t test; **P < 0.01.

It has been found that the time of onset of convulsions for control group animals was very less when compared to the extract and standard group animals. Duration of convulsions in control group animals was greater when compared to the extract and standard group animals. All the three doses of EEGH provided significant protection to mice from convulsions induced by PTZ in a dose-dependent manner.

Albino mice pretreated with EEGH exhibited significant and dose-dependent antiepileptic activity (p<0.01) and more percentage inhibition at both the doses of 30 mg/kg and 100 mg/kg when compared to Phenobarbitone sodium 40 mg/kg, i.p. (50.23%, p<0.01).

Isoniazid (INH) induced convulsions in mice

The latency of convulsions for control group mice was 25.21±0.35 min. Albino mice pretreated with EEGH showed the latency of convulsions of 35.44±0.02, 41.08±0.05 and 46.23±0.05 min (p<0.01) respectively at the doses of 10, 30 and 100 mg/kg, p.o. The standard group mice (diazepam 4 mg/kg, i.p.) showed 63.27±0.13 min (p<0.01) (Table 3).

Table 3: Effect of EEGH on Isoniazid (INH)-induced convulsions in mice

Group (n=6)	Treatment	Latency of convulsions (min)
I	DMSO	25.21±0.35
II	EEGH (10 mg/kg)	35.44±0.02**
III	EEGH (30 mg/kg)	41.08±0.05**
IV	EEGH (100 mg/kg)	46.23±0.05**
V	Diazepam (4 mg/kg, i.p.)	63.27±0.13**

EEGH: Ethanol extract of leaves of *Gossypium herbaceum*; Values are mean ± SD (n = 6). Statistical significance was determined by ANOVA, followed by Dunnett's t test; **P < 0.01.

The latency of convulsions for control group animals was very less when compared to the extract and standard group animals. All the three doses of EEGH showed the latency time more than that of control group animals and less than that of standard group animals i.e., diazepam. It has been found that all the three doses of EEGH significantly delayed the latency of convulsions in mice but failed to protect the mice against mortality.

CONCLUSION

The present investigation concluded that EEGH exhibited significant and dose-dependent antiepileptic effect potent than Diazepam and PS which may be due to raised seizure threshold or enhanced GABAergic neurotransmission by increasing GABA levels in brain.

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